Development of Tannic acid/Al (III) Nanoparticles for Controlled Release of Liraglutide: Preparation and *In Vivo* Evaluation

by

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Abstract

Liraglutide is a GLP-1 agonist recently approved for treating Type-II diabetes. Its clinical application for treating Type II diabetes has been very limited due to its short half-life time of around 13 h. In this study, we developed a ternary component nanoparticle platform of liraglutide/tannic acid/Al^{3+} for sustained release of liraglutide, where tannic acid and liraglutide form complexes and Al^{3+} served as an additional crosslinking agent. Liraglutide was encapsulated with a high efficiency (98%) and size uniformity of nanoparticles with a z-average size of 55 nm and a PDI of 0.16 using flash nanocomplexation (FNC) method. This method enables the turbulent mixing that prevented the aggregation of nanoparticles during the preparation. The optimized nanoparticles showed a sustained release profile for 7 days *in vitro* and 6 days *in vivo* following intraperitoneal injection; and it was capable of suppressing the glucose level of T2D mice under 50% of original level for 84 hours. Notably, by controlling the amount of tannic acid incorporated in the nanoparticles, the release behavior was tunable and led to significant difference of treatment outcomes in T2D mice. The scalable production capability of this manufacturing process and tunable release profile render these liraglutide/tannic acid/Al^{3+} ternary nanoparticles a promising system in future clinical translation.

Keywords: Liraglutide; Controlled release; Flash nanocomplexation; Type-II diabetes

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Thesis reader: Professor Hai-Quan Mao

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1. Introduction

Diabetes mellitus, a chronic disease with 415 million adult patients globally, has become the ninth major cause of death, in which 90% of cases are type 2 diabetes (T2D) [1]. Compared with non-diabetic counterparts, patients with T2D suffer from higher risk of cardiovascular disease (CVD), which accounts for the largest proportion (80%) of deaths cause in T2D [2, 3]. Therefore, cardiovascular (CV) safety has been listed as a criterion in the assessment for anti-diabetic agents. As increasing number of new anti-diabetic drugs are entering clinical trials, cardiovascular benefits are receiving attention as a trend of drug assessment. It requires anti-diabetic drugs not only to manage blood sugar, but also to improve cardiovascular functions. Liraglutide (brand name Victoza), as the only glucagon-like peptide-1 (GLP-1) drug tagged by FDA with specific label indicating CV benefits, is approved to reduce the risk of major CV events including cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke in T2D patient groups [4]. However, clinical application of liraglutide is hindered due to its high dose frequency (1 dose/day) and short half-life time (13 h), compared with other long-acting GLP-1 anti-diabetic drugs like dulaglutide (brand name Trulicity), with dose frequency of 1 dose/week. In order to prolong the acting time, pharmaceutical excipients are applied to dosage form. A microsphere formulation for extended release of exenatide (brand name Bydureon) has been approved by FDA in 2017.

Some progress, especially formulations for sustained release, has been made to enhance the clinical outcomes of liraglutide [5-10]. Semaglutide (brand name Ozempic), a chemical modified version of liraglutide developed by Novo Nordisk, successfully prolonged the half-

life time to 46 h at a cost of 3-fold decrease in affinity with GLP-1 receptor[10]. In addition, following the microsphere formulation of exenatide, liraglutide was similarly encapsulated in the poly(lactic acid-co-glycolic acid) microparticles, achieving a 20-day release[9]. However, it suffered from relative low encapsulation efficiency (<80%) and complexed preparation, which pose challenge on industrial translation. Besides beads and particles, hydrogel was also applied to long-term release of peptide. Injectable polymeric solution carrying liraglutide formed in-situ hydrogel in vivo after subcutaneous (s.c.) injection and made a sustained release of drug [6]. Nevertheless, the degradation time of the hydrogel lags 2 to 3 times longer than the drug-release time, which leads to accumulation of the left-over materials, and posed a challenge to multiple-dose administration in a long-term treatment.

In this study, we aimed to provide a nanoparticle (NP)-based therapeutic formulation, which combines the following advantages: (i) controlled release of liraglutide with tunable release durations; (ii) facile and scalable process with high reproducibility; and (iii) feasibility of multiple injections for potential clinical application. We designed a tannic acid (TA)/liraglutide (Lira)/Al^{3+} ternary system where peptides and TA form hydrogen bonding and Al^{3+} serves as a crosslinker by complex coordinate bonding with TA. TA is a natural plant polyphenol with pKa around 6, is approved by FDA to be applied as food additives. It forms complex with proteins and peptides based on strong interaction of hydrogen bonding. We assumed that the complex state extended the half-life time of Lira [11]. In subcutaneous and intraperitoneal environment, where pH is around 7.4, the interaction is weakened due to ionization of phenol groups on TA, thus leading to release of Lira. To generate uniform sized NPs, we use the flash

nanocomplexation (FNC) technique, where TA, Lira and Al^{3+} are mixed rapidly through turbulent flow formation (the characteristic mixing time < 50 msec). This mixing method enables homogeneous formation of NPs in a confined impinging jet (CIJ) chamber. We highlighted and analyzed the role of FNC involved in the particle assembly and the benefit it brought to preparation. Besides the prolonged release, we successfully controlled the release behavior by regulating the component amount of TA and the assessed performance of different formulations in managing blood glucose level of T2D animal model.

2. Materials and methods

2.1 Materials

Liraglutide was purchased from Taiye Biopharmaceuticals Co. Ltd. (China). Tannic acid and aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) were purchased from Sigma Aldrich. Vivaspin® 500 ultrafiltration tubes (300kDa MWCO) were purchased from Sartorius (USA). Float-A-Lyzer® G2 Dialysis Tube (300kDa MWCO) was purchased from Spectrum Labs (USA). NanoOrange Protein Quantitation Kit was purchased from Thermo Fisher Scientific (USA). Cyanine 7.5 (Cy 7.5) NHS ester was purchased from Lumi-Probe (USA). NanoOrange Protein Quantitation Kit was purchased from Thermo Fisher Scientific (USA).

2.2 FNC setup and preparation of Lira-loaded nanoparticles

A three-inlet CIJ mixer was configured as the platform for FNC (Fig. 3A). Controlled by digital syringe pumps (New ERA, NE-4000, USA), solution of Lira, TA and Al^{3+} were injected into the CIJ mixer through three inlets simultaneously, with the same flow rate. The front cut fraction

of NPs solution (1 ml) was discarded, which may contain nonuniformly mixed components. Working solutions were prepared as follows: (i) Lira was dissolved in deionized (DI) water at a concentration of 1 mg/mL and titrate to pH 7.4 with 10 mM NaOH; (ii) In case of metallic hydrolyzation, $AlCl_3 \cdot 6H_2O$ solution was generated by diluting the primary solution (1 mg/mL, pH 2.0) with DI water to the concentration of 0.05 mg/mL, followed by pH regulation to 2.0. (iii) TA was dissolved in 50 mM HEPES (pH=5.0) at concentration of 0.5, 1.0 and 2.0 mg/mL. Formulations with TA concentration of 0.5, 1.0 and 2.0 mg/mL were referred to as NPs-1, NPs-2 and NPs-3 respectively.

2.3 Nanoparticle characterization

Size distribution, polydispersity index (PDI) and zeta-potential of NPs were characterized by dynamic light scattering (DLS) and phase analysis light scattering (PALS) (Zetasizer Nano ZS, Malvern). The morphology was characterized by transmission electron microscopy (TEM) (Tecnai 12, FEI). Encapsulation efficiency was measured by ultrafiltration and protein quantitation kit. Using an ultrafiltration tube (MWCO 300 kDa), NPs and unencapsulated Lira were separated by ultrafiltration at 300×g for 20 min at room temperature. Free Lira was collected in the subnatant and measured for concentration by protein quantitation kit with an intensity-concentration standard curve of Lira. Similarly, for checking the pass ratio (PR) of the ultrafiltration device, the NPs solution was replaced by free Lira at one third of concentration of working solution followed by same protocol. The encapsulation efficiency was calculated as follows.

$$\mathrm{PR}\% = \frac{[Lira\ passed\ through\ the\ membrane]}{[Lira\ of\ working\ solution]/3}$$

$$\mathrm{EE}\ \% = (1 - \frac{[Lira\ in\ subnatant]PR}{[Lira\ of\ working\ solution]/3}) \times 100\%$$

2.4 FT-IR analysis of the interaction between components

All the suspensions were lyophilized to make solid dry sample. Mixed with KBr, solid samples were grinded thoroughly before pressing into pellets. Pressed discs were analyzed in at 20 scans per sample from 500 to 4000 cm−1.

2.5 *In vitro* release profile of liraglutides

The dialysis tube was placed in 50 ml centrifuge tube with 9 ml 1×PBS (0.05% w/v sodium azide), where the membrane part was immersed in the media. Following that 1 ml sample was pipetted into the dialysis tube, 50 ml centrifuge tube was sealed and incubated at 37 ℃ on a shaker at 100 rpm. Release samples (200 μL) were collected at specific time point of 1, 2, 3, 4, 5, 6, 7, 8-day, followed by refilling fresh media (200 μL) for maintenance of the media volume. The concentration of collected samples were determined by protein quantitation kit. The accumulative release at n-th point was calculated as follows.

$$\text{Accumulative Release}\ \% = \frac{[Lira]_n \times 10\ \text{mL} + \sum_{k=1}^{n-1}[Lira]_k \times 0.2\ \text{mL}}{[Lira]_0 \times 1\ \text{mL}} \times 100\%$$

2.6 *In vivo* biodistribution and retention

Cy-7.5 labelled Lira was introduced to NPs preparation, following the formulation of NPs-2

and NPs-3 (TA 1 or 2 mg/mL; Al^{3+} 0.05 mg/mL; Lira 1 mg/mL). Total of 200 μL NPs suspension or 200 μL free Cy 7.5- Lira was administrated on T2B mice through intraperitoneal (i.p.) injection at dose of 500 μg/kg. The distribution and intensity of Cy7.5-Lira was tracked by IVIS image system (Progama, US) at ex 780 nm and em 810 nm at post-injection timepoint of 0.5, 1, 3, 6, 24, 48, 72, 96, 120 and 144 -h. Correspondingly, around 200 μL blood sample was taken from canthus venous plexus right after imaging and 150 μL of sample was pipetted into 96-well plate. Similarly, the sample-loaded plate was imaged by IVIS image system (ex 780 nm; em 810 nm).

2.7 Potency of nanoparticles on managing glucose level

The efficacy of Lira-loaded NPs was evaluated by glucose level monitoring in T2D mice. T2D mice, which are set by leptin receptor-deficiency, are randomly divided into 4 groups with 5 mice in each. These groups were injected with low dose of free Lira solution (2 mg/kg), high dose of free Lira solution (10 mg/kg), high dose of NPs-1, 2, 3 (10 mg/kg) and 1×PBS respectively. Another group of healthy mice were set as the control group. At specific timepoints of 6, 12, 24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144, 156-h post injection, blood from tail vein were sampled and measured for glucose concentration through blood glucose meter (OneTouch® UltraVue, Johnson, USA).

2.8 Statistical analysis

All values are expressed as mean ± S.D. Comparisons among all groups were evaluated using One-way ANOVA or t-test by Graph-Pad Prism version 5.0 for Windows (GraphPad Software,

US), and $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1 Assembly of nanoparticles involves flash nanocomplexation (FNC)

Polyphenol-protein (peptide) interactions has been widely studied due to their application in food industry and biomedical field [12-15]. In most cases, protein and polyphenol form insoluble complex, leading to macroscopic precipitation in mixed solution. The phenomenon of aggregation was attributed to the (i) change of microenvironment of residues on periphery side of proteins or peptides; and (ii) crosslinking between complexes by hydrogen bonding. The non-covalent interaction between polyphenol and peptide, which involves hydrogen bonding and Van der Waals force, damage of hydration shell without change of secondary structure and exposure of hydrophobic domain of peptides[16]. Therefore, driven by hydrophobic force thermodynamically, protein-polyphenol complexes tend to aggregate. Further, complexes continuously crosslink due to the interaction between excess phenol groups and peptides that are not saturated by hydrogen bonds yet.

However, the complex can be dissociated with the change of conditions like pH and temperature. Tannic acid (TA), a natural polyphenol with pKa around 6, forms strong interaction with peptide in acidic condition (pH<6), but in human body with pH of 7.4, the partial ionization of phenol groups weaken the hydrogen bonding system and allow for peptide escape[17].

With the assumption above, we aim to design an injectable system for control release of

Liraglutide (Lira) based on TA-peptide interaction, whereas the natural property of aggregation of TA-Lira complex pose great challenge on injectability. To address it, we introduced the Al^{3+} in nanoparticles preparation, which forms coordinate bonds with TA[18]. Importantly, this coordinate reaction, which is common for trivalent metal ions, gives transparent solution and does not induce aggregation. Serve as a surfactant, Al^{3+} ions 'quench' the excess phenol groups and thus keep the complexes from further crosslinking.

$$6\ PhOH + M^{3+} \longrightarrow H_3[M(OPh)_6] + 3H^+$$

For severing better as a role of surfactant, Al^{3+} were required to be mixed with TA and Lira homogenously. To achieve this, we utilized the FNC technology, where all the solutions of components are introduced into micro mixing chamber with high flow rate. As a technology generating turbulence for rapid and homogenous mixing, FNC has been broadly applied in nanoparticles preparation[19-21]. Based on thermodynamic availability, through FNC, we aim to optimize and control the process of assembly kinetically. Low flow rate of 1 ml/min leads to heterogenous mixing, thus causing multiple species of components in the solution. It was observed by DLS spectrum of multiple peaks range from 43.7 nm to 5030 nm (Fig. 2A). The 5030 nm peak, which indicates aggregation, was resulted from absence of Al^{3+} in some local regions during the mixing. With the increase of flow rate, the condition of mixing got improved and the possibility of Al^{3+} being evenly dispersed increased greatly. The micro-sized peaks faded with growth of flow rate and got disappeared when it reached 20 mL/min. The main peak, which stably remained at around 55 nm, indicated that the assembly of desirable nanoparticles may depend on mostly by thermodynamic. The result of TEM further confirm the phenomenon

observed by DLS (Fig. 2B). Significant difference of homogeneity prepared at different flow rate (1, 10, and 25 mL/min) can be observed. The aggregation (crosslinked dark substance) existed in sample of 1 and 10 mL/min, but not in that of 25 mL/ml.

3.2 Controlled release of liraglutide from nanoparticles

Due to the strong interaction between TA and peptides, Lira was successfully encapsulated at an ideal encapsulation efficiency of 98%. To demonstrate the interaction between components, FT-IR was operated and the change of shape and position of peaks was observed. Affected by the interaction between TA with Lira or Al^{3+}, the wide peak at 3391.8 cm^{-1}, indicating the phenol groups on TA, changed the shape and shift to wavenumber of around 3415 cm^{-1} in both cases, which verified the hydrogen and coordinate bonding on phenol groups (Fig. 3A).

As a critical factor in drug release, the amount of surface area of nanoparticles needs to be guaranteed for a sustained release. To maintain the surface area, it requires that nanoparticles should have stable size and avoid aggregation *in vivo*. To measure that, we place the NPs suspension in 1×PBS and monitor the size change for 7 days. No dramatic change was observed from DLS in all three formulations that the size change was within the range of 10% over 7 days (Fig. 3B), where the negative surface charge (-26.1±0.8 mV) may contribute to the size stability.

Based on the understanding of the release mechanism that hydrogen bonding is weakened by partial ionization on phenol groups of TA, we aimed to control the release rate by tuning the

amount of TA. From the *in vitro* release study, all the formulations achieved sustained release, and furthermore, the different formulations show obviously different release behavior, including different amount of burst release and sustained release rate (Fig. 3C). With highest amount of TA (2 mg/mL), NPs-3 shows lowest burst release of 12% and slowest sustained release (60 % released for 8 days). We attributed it to the high density of phenol groups, which trap the Lira and prevent the release. On the other hand, as expected, formulation with lowest amount of TA (0.5mg/ml, NPs-1) showed huge burst release of 30 % and 80 % got released over 8 days. This capacity of release regulation indicates the great potential that this ternary system may satisfy the different requirements for drug release rates in clinical trails.

3.3 *In vivo* biodistribution and retention

To further confirm the release behavior, biodistribution and retention *in vivo* was analyzed and it showed the similar trend to the situation *in vitro*. Besides fluorescence-based animal imaging, blood sample was also collected for assessment of the content of Lira in circulation. The signal for free Cy-7.5 labelled Lira reached the peak at 3 h and attenuated rapidly within 24 h, which can be observed from both animal imaging and blood imaging (Fig. 4A). After 48 h, the signal was weak in animal imaging and can be hardly detected in blood sample, indicating the quick degradation and body clearance of free Lira. In contract, NPs formulation with same dose of Lira showed longer retention time. For signal of NPs-2, It took 120 h to fall to the same level as free Lira did at 24 h and for NPs-3, it took even longer time. Notably, the burst release within 1 h in NPs-3 is less than that in NPS-2, which can be observed from the blood sample and signal quantification (Fig. 4B; Fig. 4C). Interestingly, in animal imaging of NPs formulation, there is

certain degree of enhancement of total signa, compared with the starting time point (Fig. 4C). We attribute this unexpected phenomenon to the fluorescence quench that the Cy-7.5 groups on Lira quenched at high concentration inside nanoparticles. With the proceeding of release, these quenched Cy-7.5 groups were activated again when escaping from the NPs. Nevertheless, the process of drug diffusion can still be monitored from animal imaging and the retention time can be assessed from blood sample. Although the duration of release was different between *in vivo* and *in vitro* due to the complexity in *In vivo* environment, trends of release behavior, including difference of burst release amount and sustained release rate among formulations, match well with each other.

3.4 Treatment potency of nanoparticles on managing glucose level

Corresponding to the biodistribution and drug retention study, treatment efficacy was studied on db/db model mice for analyzing the practical potency of different NPs formulations. Db/db mice were administrated with NPs-1, 2 and 3 at a single dose of 10 mg/kg respectively. Two positive control groups were set by injection with free Lira (2 mg/kg; 10 mg/kg) and blank control was set by injection with PBS. For all the drug treated groups, glucose level decrease rapidly to normal level within 6 h, where the burst release of NPs formulation guaranteed the effective concentration (Fig. 5A). In both positive control groups, glucose level began to rebound at very early timepoints of 6-h post injection and recover to original level within 48 h. Although the high dose of free Lira (10 mg/ml) leaded to a half-recovery time of more than 24 h, it was shorter than NPs formulation, especially compared with NPs-3. For NPs formulations, NPs-3 revealed the most sustained release with half-recovery time of 84 h. It took glucose level

more than 60 h to start to rebound and more than 150 h to recovery to original level. As expected, NPs-1 and NPs-2, the formulations with faster release, made glucose level recover to 50% of origin by shorter time of around 30 and 40 h respectively. The differences of treatment outcome among NPs formulations and the different retention time in circulation mutually confirmed and it indicate the capacity for tuning release behavior both *in vitro* and *in vivo*.

Also, weight change was monitored during the treatment, since Lira is also a GLP-1 drug approved to treat obesity (Fig. 5B). From the data, all the treated groups showed weight control potency and compared with free Lira group, NPs-1 and NPs-2 revealed slight advantage. Remarkably, unlike other groups, NPs-3 helped maintain the decreased body weight in the first 4 days, indicating complete containment on body weight. It may be attributed to the most sustained release behavior of NP-3.

4. Conclusion

Here we report a nanoparticle platform for prolonged and controlled release of therapeutic peptides, composed of tannic acid, peptides and trivalent ion. In this typical ternary system, hydrogen bonding between tannic acid and peptides serve as the major interaction in particle forming and aluminum ions serve as surfactant. The technology of flash nanocomplexation (FNC) played an important role in nanoparticle preparation through kinetic control, where rapid homogeneous mixing prevent aggregation and thus made formulations injectable. By in vitro release study and retention study in vivo, this system showed great capacity of sustained release and furthermore, tunable release behavior. Based on convincible mechanism of release that

ionization of phenol groups on tannic acid weakened the hydrogen bonding, amount of burst release and sustained release rate were controlled by tuning the component amount of tannic acid. The enhancement of drug potency in treatment study further confirm that the sustained release of this system worked well *in vivo*. Importantly, in the complexed *in vivo* condition, treatment outcome still revealed obvious difference among different formulations, which indicates the capacity of pharmacodynamics tuning *in vivo*. To sum up, this nanoparticle platform holds great potential in industrial clinical translation and worth for further study.

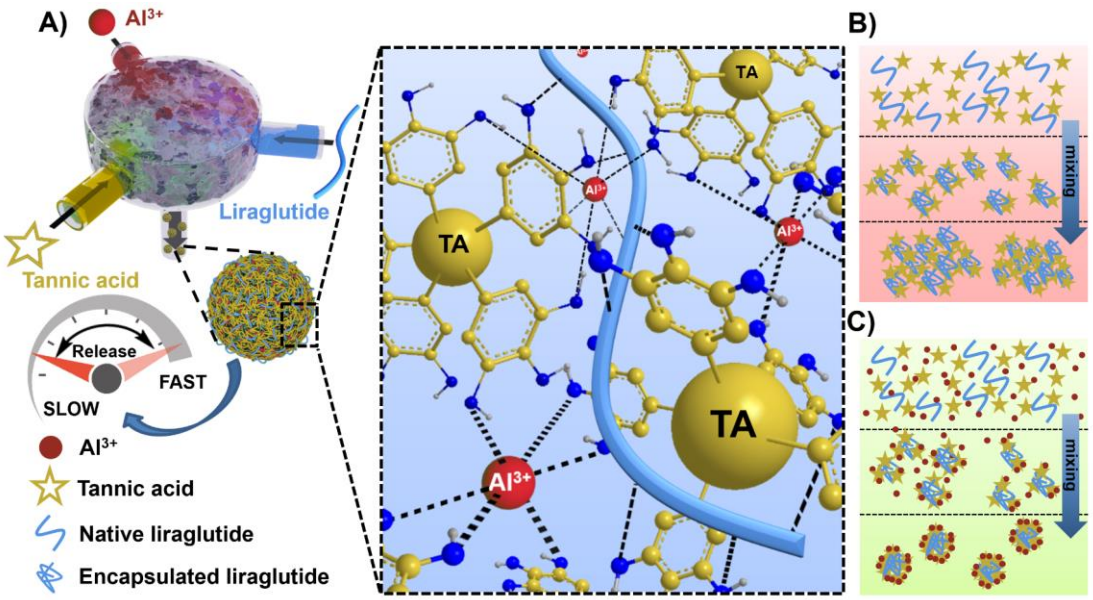

Fig 1. Schematic diagram nanoparticles and preparation. (A) Schematic diagram of Lira/TA/Al^{3+} nanoparticles preparation based on FNC technology and demonstration of inside interactions; (B) Formation of aggregation in absence of Al^{3+}; (C) Formation of uniform nanoparticles with the participation of Al^{3+}.

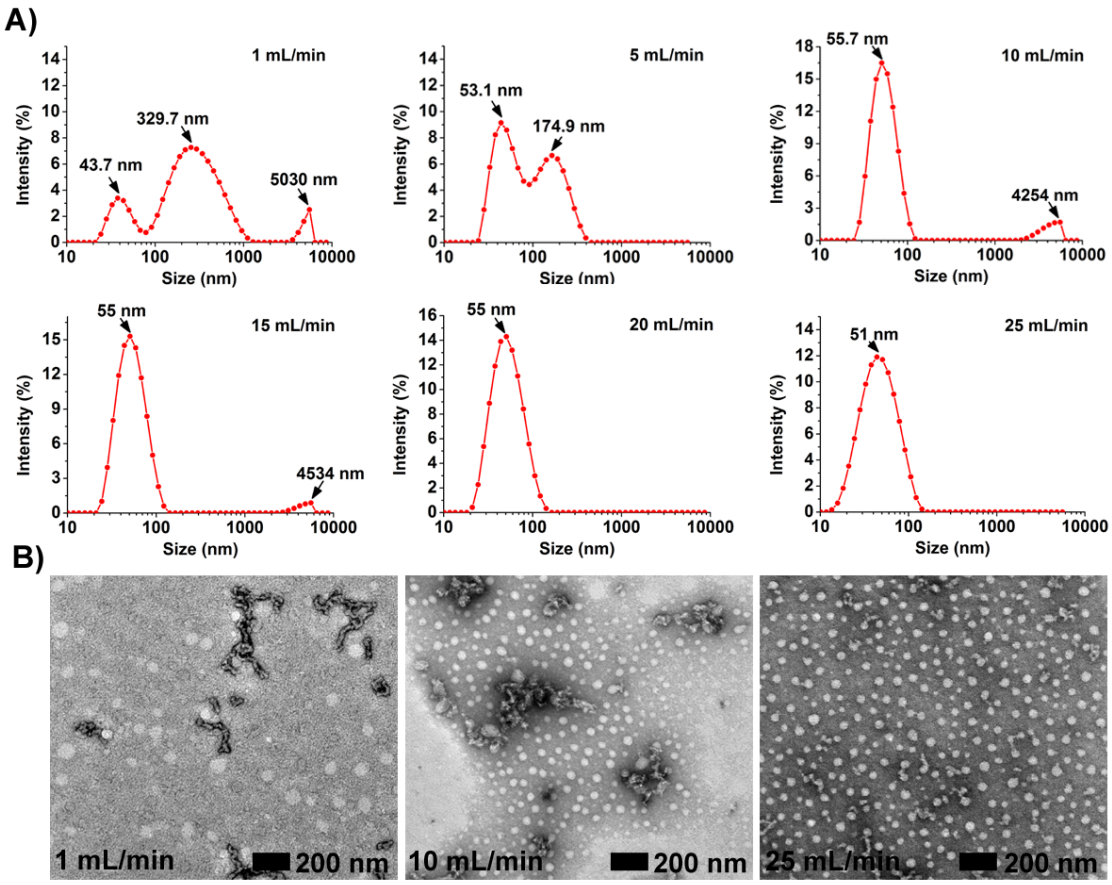

Fig 2. Size and distribution of nanoparticles. (A) Effect of volumetric flow rate of FNC on size distribution of NP-3 formulation. Curves (filled circles) and bars represent mean ± S.D. (n= 3) of measurements from three different preparations; (B) TEM images of Lira-loaded NP-3 prepared at flow rate of 1 ,10 and 20 mL/min.

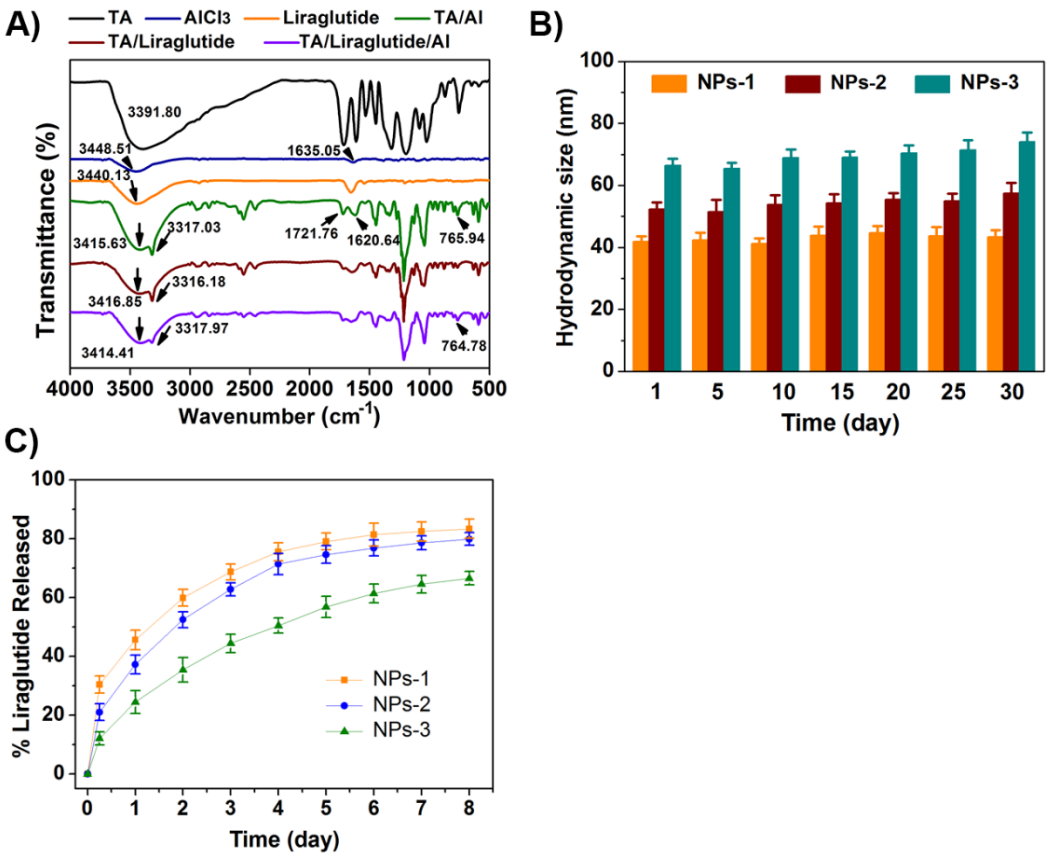

Fig 3. Characterization of Lira-loaded nanoparticles. (A) FT-IR spectrum of TA, $AlCl_3$, liraglutide, TA/Al^{3+} complex, TA/liraglutide complex and TA/liraglutide/Al^{3+} nanoparticles; (B) Colloidal stability of formulations indicated by size change over a month; (C) In vitro release profile of formulations in PBS.

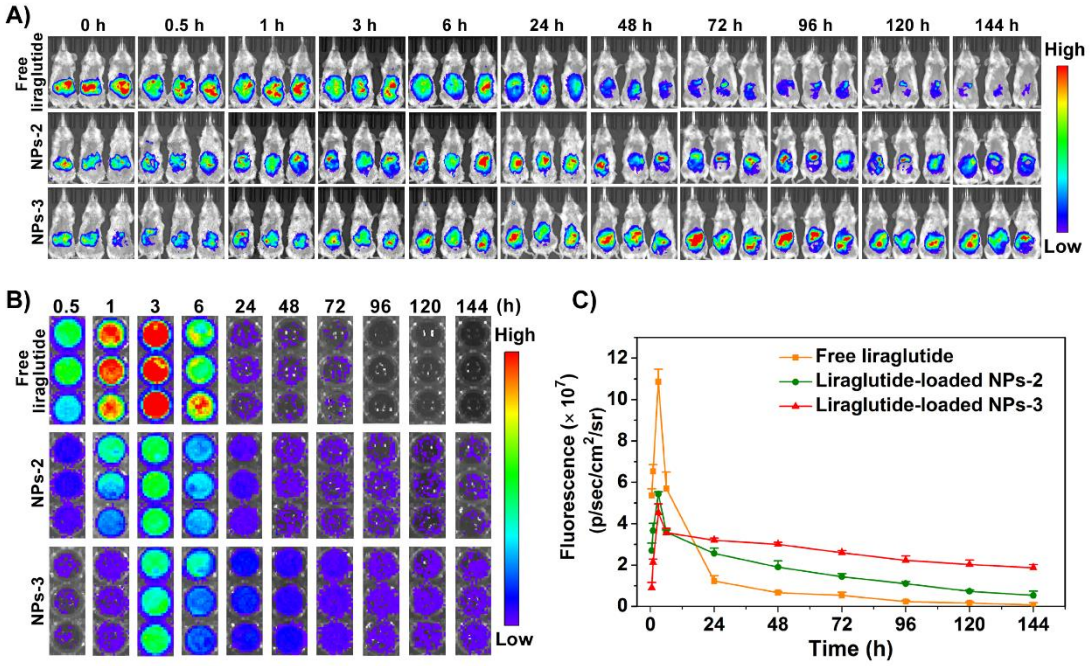

Fig 4. Sustained release of Lira from formulations in Balb/c mice post i.p. injection. (A) *In vivo* fluorescence imaging of mice at different time points following single i.p. injection of Cy 7.5-labeled free Lira and Lira loaded nanoparticles at dose of 500 μg/kg; (B) Fluorescence imaging at different time points of blood samples collected from mice administrated with Cy 7.5-labeled free Lira or Lira-loaded nanoparticles. (C) Quantification of fluorescence intensity of the Cy 7.5-labeled free Lira and Lira-loaded nanoparticles in peritoneal cavity given by IVIS images and bars represent mean ± S.D. (n= 3).

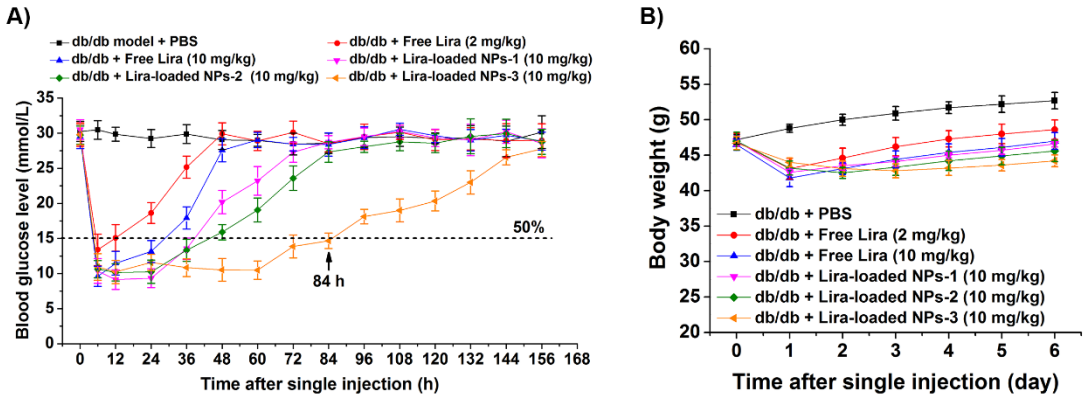

Fig. 5. *In vivo* efficacy of exendin-4-loaded nanoparitcles in db/db mice (T2D model). (A) Blood glucose levels of db/db mice following single i.p. injection of free Lira (2 mg/kg; 10 mg/kg) or Lira-loaded NP-1, 2 and 3 (Lira of 10 mg/kg). Control groups were PBS-treated; (B) Body weight monitoring of mice treated with different formulations.

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Bibliography

Jinchang Zhu was born in 1995 in China.

In 2013, Jinchang started his undergraduate study at Beijing University of Chemical Technology, majored in Polymer Materials and Engineering. During this time, he worked with Dr. Li Wang and did research on free radical living polymerization and functional polymer.

In 2017, Jinchang started his master's study at Johns Hopkins University and joined in Dr. Hai-Quan Mao's research group. During the two-year research at Institute for NanoBioTechnology, he did research on nanomedicine and therapeutic delivery.

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EDUCATION BACKGROUND

Beijing University of Chemical Technology Beijing, China
Major in Polymer Materials and Engineering, Bachelor of Engineering 09/2013-07/2017

Johns Hopkins University Baltimore, U.S.
Major in Materials Science and Engineering, Master of Science 09/2017-06/2019

PUBLICATION

Zhiyu He, Yizong Hu, Tianqi Nie, Haoyu Tang, **Jinchang Zhu**, Kuntao Chen, Lixin Liu, Kam W, Leong, Yongming Chen, Hai-Quan Mao "Size-Controlled Lipid Nanoparticle Production Using Turblent Mixing to Enhance Oral DNA Delivery", *Acta Biomaterialia*, **doi: https://doi.org/10.1016/j.actbio.2018.09.047**;

Zhiyu He, Yizong Hu, Zaizhi Gui, Yang Zhou, Tianqi Nie, **Jinchang Zhu**, Zhijia Liu, Kuntao Chen, Yongming Chen, Kam W Leong, Peng Cao, Lixin Liu, Hai- Quan Mao "Sustained Release of Exendin-4 from Tannic Acid/Fe (III) Nanoparticles Prolongs Blood Glycemic Control in a Mouse Model of Type II Diabetes", *Journal of Controlled Release,* **doi: https://doi.org/10.1016/j.jconrel.2019.03.014**;

J. Zhu, L. Wang, W. Yang "Terpolymer of styrene, maleic anhydride and a styrenic monomer containing a tetraphenylethylene moiety prepared by RAFT polymerization and its aggregation induced emission behavior", *The 2017 National Polymer Academic Papers Conference* (10.10.2017, Chengdu, China), Abstract ID: 618409.

RESEARCH & PROFESSIONAL EXPERIENCE

Research Assistant 09/2017- 06/2019
Institute for NanoBioTechnology (INBT), Johns Hopkins University
Advisor, Prof. Hai-Quan Mao, Associate Director, INBT; Associate Editor, Biomaterials
Research Projects:

- Size-Controlled Lipid Nanoparticle Production Using Turbulent Mixing to Enhance Oral DNA Delivery
  - Developed a scalable flash complexation method to produce, purify and concentrate lipid nanoparticles with various structures (lipo-polyplex and lipo-complex), controlled size and surface charge;
  - Designed, optimized and characterized the DOTAP/DNA lipo-complex formulations to have stability in simulating gastrointestinal fluids with various pH;
  - Conducted *in vitro* transfection experiments to compare transgene activities of different formulations;
  - Assisted *in vivo* biodistribution and transfection experiments to examine delivery efficiency and transgene activity.
- A facile Strategy to Enhance the Brain Targeting Delivery for Alzheimer's Disease Therapy
  - Assisted with nanoparticle preparation for levodopa delivery into brain
  - Studied the effect of nanoparticle size on the brain-specific delivery efficiency;
  - Optimized and characterized the TA/PVA/levodopa formulation with controlled particle size (50 nm);
  - Operated the animal surgery and MRI assistance to confirm that nanoparticles can be delivered to ventricle.
- Effect of the nanoparticle size of aluminum adjuvant on immuno-activation
  - Produced size-controlled aluminum nanoparticles using flash complexation technique with a tunable size range (70~200 nm) to examine its effect on immune-activation;

- Optimized and characterized different formulations including OVA/$Al(OH)_3$, Oleic acid/$Al(OH)_3$ and Oleic acid/OVA/$Al(OH)_3$. Achieved nanoparticle stability in PBS suspension for maintaining the size upon dosing to cells in their culture medium and subcutaneous injections;
- Carried out cell uptake and confocal study for confirmation of the size effect on the immune-activation of dendritic cells and macrophages.

Research Assistant, 03/2016-08/2017
Organic Materials Surface Science and Engineering Lab, Beijing University of Chemical Technology (BUCT)
Advisor, Professor Wantai Yang, Director, Beijing Laboratory of Biomedical Materials, Academician of Chinese Academy of Sciences; and Dr. **Li Wang, Associate Professor, BUCT**

Research Projects:

- RAFT Polymerization of Terpolymers of Styrene, Maleic Anhydride and Styrene Containing a Tetraphenylethylene Moiety and Its Aggregation-Induced Emission Behavior
  - Synthesized the terpolymer with pH-depended aggregation Induced emission behavior based on maleic anhydride and styrene derivatives;
  - Designed and synthesized the VBO-TPE monomer and conducted RAFT polymerization; obtained well-controlled the molecular weight and PDI of the terpolymer with AIE group side chains;
  - Carried out the characterization of monomer and polymer and study of pH-depended aggregation Induced emission behavior to explore the potential application in biosensors.